Risk assessment of new sequencing information on GM maize event DAS-59122-7

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Abstract: The EFSA Panel on Genetically Modified Organisms (GMO Panel) has previously assessed genetically modified (GM) maize DAS-59122-7 as a single event as well as part of several stacked events. These maize events were found to be as safe as their conventional counterparts and other appropriate comparators with respect to potential effects on human and animal health, and the environment. On 26 July 2016, the European Commission received from Pioneer new nucleic acid sequencing data on maize event DAS-59122-7 and updated bioinformatic analyses using the new sequencing data. The European Commission tasked EFSA to analyse these data and to indicate whether the previous conclusions of the GMO Panel on the previously assessed GM maize events remain valid. The GMO Panel used the appropriate principles described in its guidelines for the risk assessment of GM plants to analyse the received data. The new sequencing data indicated three base pair (bp) differences compared to the sequencing data originally provided; two located in a non-coding region of the insert and one located in the 5 genomic flanking region. These base pairs reported as differences in the new nucleic acid sequencing data on maize event DAS-59122-7 had already been present in the original plant material used for the risk assessment. Thus, with the exception of bioinformatics analyses, the studies performed for the risk assessment remain valid. The new sequencing data and the bioinformatic analyses performed on the new sequence did not give rise to safety issues. Therefore, the GMO Panel concludes that the original risk assessment of event DAS-59122-7 as a single and as a part of stacked events remains valid.

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STATEMENT

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EFSA Panel on Genetically Modified Organisms (GMO),
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Abstract

The EFSA Panel on Genetically Modified Organisms (GMO Panel) has previously assessed genetically modified (GM) maize DAS-59122-7 as a single event as well as part of several stacked events. These maize events were found to be as safe as their conventional counterparts and other appropriate comparators with respect to potential effects on human and animal health, and the environment. On 26 July 2016, the European Commission received from Pioneer new nucleic acid sequencing data on maize event DAS-59122-7 and updated bioinformatic analyses using the new sequencing data. The European Commission tasked EFSA to analyse these data and to indicate whether the previous conclusions of the GMO Panel on the previously assessed GM maize events remain valid. The GMO Panel used the appropriate principles described in its guidelines for the risk assessment of GM plants to analyse the received data. The new sequencing data indicated three base pair (bp) differences compared to the sequencing data originally provided; two located in a non-coding region of the insert and one located in the 5′ genomic flanking region. These base pairs reported as differences in the new nucleic acid sequencing data on maize event DAS-59122-7 had already been present in the original plant material used for the risk assessment. Thus, with the exception of bioinformatics analyses, the studies performed for the risk assessment remain valid. The new sequencing data and the bioinformatic analyses performed on the new sequence did not give rise to safety issues. Therefore, the GMO Panel concludes that the original risk assessment of event DAS-59122-7 as a single and as a part of stacked events remains valid.

Keywords: GMO, maize (*Zea mays*), DAS-59122-7, nucleotide sequence, Regulation (EC) No 1829/2003

Requestor: European Commission

Question number: EFSA-Q-2016-00506

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Table of contents

1. Introduction

Genetically modified (GM) maize event DAS-59122-7 was developed to confer resistance to certain coleopteran species, such as the corn rootworm larvae (*Diabrotica* spp.), and also to confer herbicide tolerance to glufosinate ammonium-based herbicides. This is achieved by the expression of the *cry34Ab1* and *cry35Ab1* genes derived from *Bacillus thuringiensis* (strain PS149B1) that encode for the insecticidally active Cry34Ab1 and Cry35Ab1 proteins and by the expression of the *pat* gene that produces the herbicide-tolerant phosphinothricin acetyltransferase (PAT) enzyme.

The EFSA Panel on Genetically Modified Organisms (GMO Panel) has previously assessed maize event DAS-59122-7 as a single event as well as part of stacked events (see Table 1).

Table 1: GMO Panel scientific opinions on maize event DAS-59122-7 (59122)

Event	Application*	EFSA Scientific Opinions
59122	EFSA-GMO-NL-2005-12	EFSA (2007)
1507 × 59122	EFSA-GMO-NL-2005-15	EFSA (2009a)
59122 × NK603	EFSA-GMO-UK-2005-20	EFSA (2008)
59122 × 1507 × NK603	EFSA-GMO-UK-2005-21	EFSA (2009b)
59122	EFSA-GMO-NL-2005-23	EFSA GMO Panel (2013)
MON 89034 × 1507 × MON 88017 × 59122	EFSA-GMO-CZ-2008-62	EFSA GMO Panel (2010a)
Bt11 × 59122 × MIR604 × 1507 × GA21	EFSA-GMO-DE-2011-99	EFSA GMO Panel (2016)

*: With the exception of EFSA-GMO-NL-2005-23 that is for cultivation, the scope for all other GM applications is for import and processing.

1.1. Background and Terms of Reference as provided by the requestor

On 26 July 2016, Pioneer sent to the European Commission new sequencing information relating to maize event DAS-59122-7, on the basis of Articles 9 and 21 of Regulation (EC) 1829/2003. On 29 July 2016, the European Commission requested the European Food Safety Authority (EFSA) to evaluate the data and analyses provided by Pioneer and indicate whether, on the basis of these elements, the conclusions of adopted opinions for maize DAS-59122-7 as a single event or as part of stacked events, have to be adapted. Subsequently, the GMO Panel has evaluated the data and methodology provided for maize event DAS-59122-7 and considered these elements in the context of previous conclusions.

2. Data and methodologies

2.1. Data

The applicant followed the relevant parts of the GMO Panel guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011) to investigate the insert sequence and to perform the bioinformatics analyses. In delivering this statement, the GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants (EFSA GMO Panel, 2011).

2.2. Methodologies

In delivering this statement, the GMO Panel took into account information provided by the applicant and relevant scientific publications.

2.2.1. Sequence information previously submitted to EFSA for maize event DAS-59122-7

The applicant had previously submitted information on the sequence of maize event DAS-59122-7, as part of application EFSA-GMO-NL-2005-12.[1] Maize event DAS-59122-7 consists of one insert comprising three expression cassettes; the *cry34Ab1* cassette which contains the following elements: the *ubi1ZM* promoter of the polyubiquitin gene from *Zea mays*, the synthetic, maize-optimised *cry34Ab1* gene, based on the *cry34Ab1* gene from *B. thuringiensis* (strain PS149B1) and the *pinII* terminator sequence of the proteinase inhibitor II gene from *Solanum tuberosum*; the *cry35Ab1*

[1] Annex 7, technical dossier (EFSA-GMO- NL-2005-12, confidential information).

cassette which contains the following elements: the TA peroxidase promoter of the peroxidase gene from *Triticum aestivum* (wheat), the synthetic, maize-optimised *cry35Ab1* gene, based on the *cry35Ab1* gene from *B. thuringiensis* (strain PS149B1) and the *pinII* terminator sequence; and the *pat* cassette which contains the following elements: the CaMV 35S promoter from Cauliflower Mosaic Virus, the synthetic, maize-optimised *pat* gene from *Streptomyces viridochromogenes* and the CaMV 35S terminator from Cauliflower Mosaic Virus. 2,593 base pair (bp) of the 5′ flanking region and 1,986 bp of the 3′ flanking regions of event DAS-59122-7 were identified.

2.2.2. New information for maize event DAS-59122-7 submitted as part of the current mandate

The applicant has recently resequenced the maize event DAS-59122-7 in a stack and compared this sequence with the original maize event DAS-59122-7 sequence reported in 2005.[2] This revealed three differences which are: two bp located in the TA peroxidase promoter of the *cry35Ab1* expression cassette at bp positions 6,526 and 6,562, and one bp in the 5′ flanking genomic region at position 1,581 (see Table 2).

Table 2: Identified differences in the sequence of the insert and flanking regions in maize event DAS-59122-7

Identified difference	Position*	Reported in 2005	Reported in 2016
TA peroxidase promoter	6,526	AAA**C**ATC	AAA**A**ATC
TA peroxidase promoter	6,562	TCC**G**ACA	TCC**A**ACA
5′ flanking region	1,581	ACT**G**CAC	ACT**A**CAC

*: Positions are relative to the complete sequence of transfer-deoxyribonucleic acid (T-DNA) insert and genomic flanking border regions originally reported for the maize event DAS-59122-7.[1]

Genomic DNA from the same maize event DAS-59122-7 material analysed in 2005 was used as a template to amplify and sequence two specific regions where the sequence differences were found. The results indicated that the three bp differences found in the stack were present in the original maize event DAS-59122-7 material.[1,2]

The applicant carried out bioinformatic analyses using the updated nucleotide sequence in order to investigate (1) if any known maize genes were disrupted by the DAS-59122-7 insert; (2) if the newly expressed protein or any other open reading frame (ORF) present within the insert or spanning the junction sites shows similarity to known allergens or toxins; (3) if the insert contains sequences that would facilitate horizontal gene transfer (HGT) to microorganisms.

3. Assessment

Based on the data provided, it can be concluded that the sequence differences found in maize event DAS-59122-7 in 2016 were already present in the original material used in the risk assessment process (EFSA, 2007, 2008, 2009a,b; EFSA GMO Panel, 2010a, 2013, 2016). Furthermore, the differences identified are located outside the coding sequences of the newly expressed proteins Cry34Ab1, Cry35Ab1 and PAT; two nucleotides are in the TA peroxidase promoter of the *cry35Ab1* expression cassette and one nucleotide is in the 5′ genomic flanking region. Therefore, only some aspects of the bioinformatics analyses needed to be updated. The other studies performed for the risk assessment are not affected by the new sequencing information. Considering the location of the three bp differences, only the analysis of ORFs containing these differences with regard to potential similarity with allergens or toxins, as well as the implications of these differences on the potential for HGT were considered relevant for the current assessment. Bioinformatic searches for similarity to allergens were performed according to EFSA guidelines (EFSA GMO Panel, 2010b, 2011). Results indicate that none of the ORFs containing the three bp changes show similarity with known allergens or toxins. Sequence analysis did not identify any similarity between the regions containing the three bp differences and microbial sequences. Therefore, these sequence differences do not affect the likelihood of HGT.

[2] Annex 11, DAS-59122-7 New Sequence Information (confidential information) and additional information: 19/9/2016 (confidential information).

4. Conclusions

Based on analysis of the data provided, it can be concluded that the sequence of the DAS-59122-7 maize event present in the original material used in the risk assessment process of maize DAS-59122-7 as a single event as well as part of stacked events already contained the nucleotide differences reported in 2016 and these differences are not located in coding regions of the insert. Therefore, studies other than bioinformatics are not affected by this new sequence information. The bioinformatic analyses performed on the corrected sequence did not give rise to safety issues. The GMO Panel concludes that the original risk assessment of maize event DAS-59122-7 as a single and as a part of stacked events remains valid.

Documentation provided to EFSA

1) Letter from the European Commission, received on 29 July 2016, concerning a request to analyse new sequencing information for maize event DAS-59122-7.

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Abbreviations

bp	base pair
GM	genetically modified
GMO Panel	EFSA Panel on Genetically Modified Organisms
HGT	horizontal gene transfer
ORF	open reading frame
PAT	phosphinothricin acetyltransferase
T-DNA	transfer-deoxyribonucleic acid